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## The Role of Carotenoids in Natural and Artificial Photosynthesis

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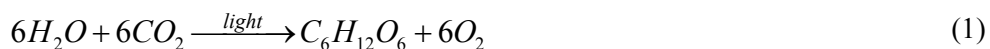
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# Introduction

Sunlight constitutes the primary source of energy for nearly all life on earth. Plants, algae and a number of bacteria directly use sunlight to sustain their lives, in a process known as photosynthesis. The photosynthetic process carried out by plants algae and certain bacteria is defined as oxygenic; in the process carbon dioxide is reduced to carbohydrates and the removal of electrons from water results in the release of molecular oxygen. The whole process can be summarized by the following deceptively simple equation:



The process of photosynthesis thus ultimately leads to the formation of stable organic compounds, and is formidably complicated. It is the result of a plethora of reactions carried out by a number of highly structured macromolecular and supramolecular devices. One of the many fascinating areas of photosynthesis research is the study of the early events, the events taking place within an antenna and reaction centre system after the absorption of a photon. Typically the lifetime of the excited state of a photosynthetic pigment is between  $10^{-9}$  and  $10^{-12}$  seconds. Yet, despite this extremely short lifetime photosynthetic antennas are extremely efficient at harvesting light and transferring the absorbed energy to the reaction centre, a photosynthetic device where the excited-state energy is converted in the form of a charge separated state. Today the advent of advanced ultrafast spectroscopic techniques has made it possible to literally see the excitation energy migrate within a photosynthetic unit and to put strong quantitative bases to the early events of the photosynthetic process.

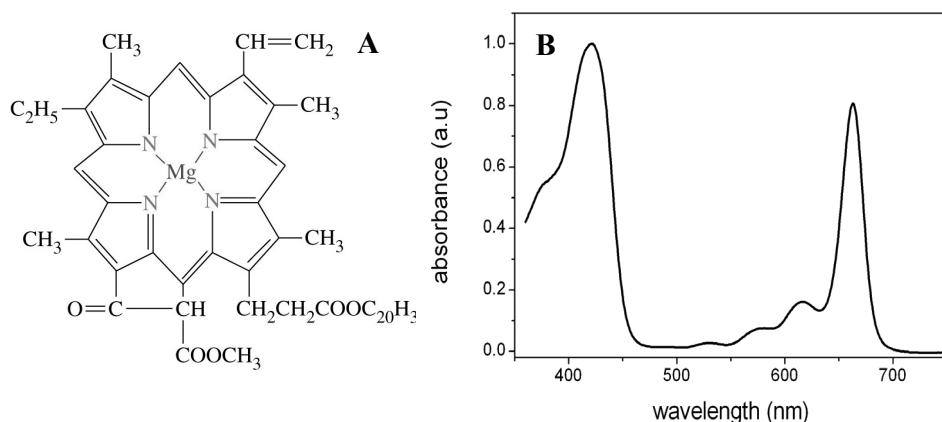
## Photosynthetic pigments

The radiant energy of the sun is efficiently collected by a number of pigments within a photosynthetic antenna. Chlorophylls are the main light-harvesting pigments in plants and cyanobacteria. Chlorophyll exists in a number of variants with different chemical stability and spectroscopic features<sup>1</sup>. Another type of pigments which are ubiquitous in photosynthetic systems are carotenoids. The common feature of most photosynthetic pigments is a network of alternating carbon-carbon single and double bonds which makes up a delocalized  $\pi$ -electron system responsible for most spectroscopic properties of the molecules.

## Chlorophylls

Chemically chlorophylls are classified as chlorins. They are characterized by a tetrapyrrole ring with a size of approximately 1x1 nm attached to phytol tail (Figure 1A). The tetrapyrrole with its delocalized  $\pi$ -electron system of alternating carbon-carbon single and double bonds, is responsible for most of the spectroscopic properties of these molecules.

Figure 1B shows the absorption spectrum of chlorophyll *a*, the most common variant of chlorophyll, dissolved in ethanol; the spectrum is characterized by a strong band peaking around 663 nm, known as the  $Q_y$  band and a smaller band at 614 nm, the  $Q_x$  band superimposed on a high-frequency vibronic progression. In the blue region the spectrum displays the absorption of the Soret band.

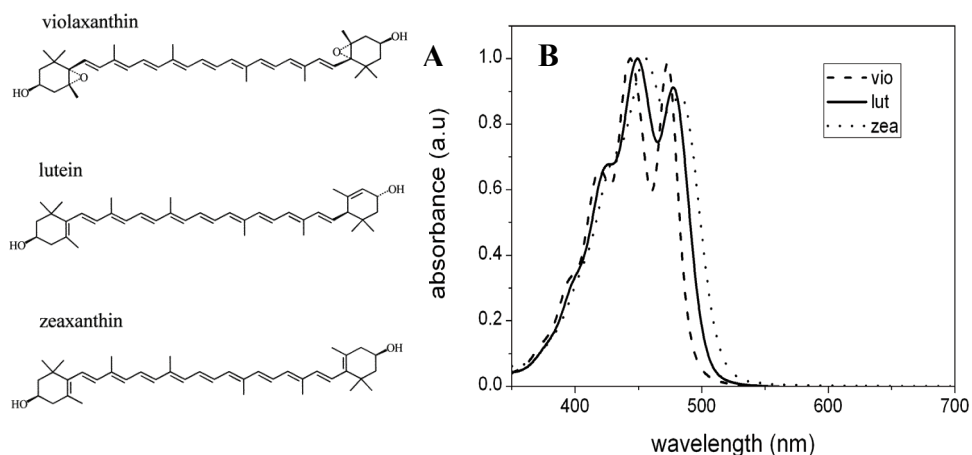


**Figure 1.** (A) The structure of chlorophyll *a*. (B) Absorption spectrum of chlorophyll *a* in ethanol.

## Carotenoids

Carotenoids are ubiquitous pigments in living organisms which are responsible for the kaleidoscope of colours found in nature; the variety of colours found in vegetables, fruit, marine organisms and birds are an example. Besides their aesthetic role, carotenoids play crucial physiological and biological roles<sup>2,3</sup>. In photosynthesis they act as accessory light-harvesting pigments; they absorb light in the blue-green region of the solar spectrum and transfer the energy to neighbouring chlorophylls, thus increasing the absorption cross section for photosynthesis. Carotenoids also play important structural roles. Of vital importance for the survival of the photosynthetic apparatus is the role of carotenoids in

photoprotection discussed below. Carotenoids are found in most antenna systems and reaction centres where they protect the system from autooxidation. Structurally carotenoids are polyenes, characterized by a linear system of alternating carbon-carbon single and double bonds. Figure 2A shows the structure of lutein, violaxanthin and zeaxanthin, three carotenoids found in the main light-harvesting antenna of green plants, the light-harvesting complex II (LHCII). Their absorption spectra in acetone are shown in Figure 2B. The strong absorption band in the visible region corresponds to the  $S_0 \rightarrow S_2$  transition. Hidden below the  $S_2$  state there are a number of hidden dark states, invisible to conventional one photon absorption spectroscopy.



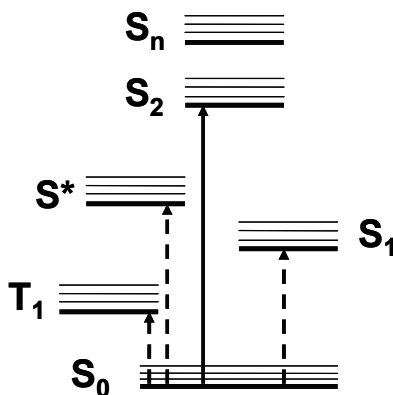
**Figure 2.** (A) The structure of three carotenoids present in LHCII, violaxanthin, lutein and zeaxanthin (only present under conditions of high light). (B) Their absorption spectra in acetone.

### The carotenoids excited state manifold

Figure 3 shows a general carotenoid excited state diagram. The strong absorption in the blue green region originates from the allowed  $S_0 \rightarrow S_2$  transition. Hidden below  $S_2$  lies a number of excited states known as dark, since they are invisible to conventional absorption spectroscopy due to symmetry reasons. The  $S_1$  state is active in energy transfer in a number of light-harvesting systems<sup>3-6</sup> and appears to be crucial for the photoprotection of the photosynthetic apparatus under conditions of excess light illumination<sup>7,8</sup>. Chapters 5 and 6 show that the  $S_1$  state can act as an energy sink by accepting energy from neighbouring chlorophyll(s) and dissipating

it as heat. In some asymmetric carotenoids, or when the molecule is in a highly asymmetric environment<sup>9</sup>, the  $S_1$  state can take up a partial charge transfer character and be referred to as  $S_1$ /ICT state<sup>7,10,11</sup>. The  $S^*$  state has been discovered in the light-harvesting complex I (LHI) of the purple photosynthetic bacterium *Rs. rubrum*<sup>12</sup> where it was shown to lead to the generation of triplet states on an ultrafast timescale. The  $S^*$  state was also shown to be active in energy transfer to bacteriochlorophyll in the LHII complex of *Rhodobacter spaeroides*<sup>13</sup>. In chapter 3 of this thesis we show that the  $S^*$  state can also act as an intermediate state in the  $S_2$  to  $S_1$  internal conversion pathway.

Below these singlet states lies the lowest triplet state, responsible for the quenching of chlorophyll triplet states and for scavenging the injurious singlet oxygen<sup>2</sup>.



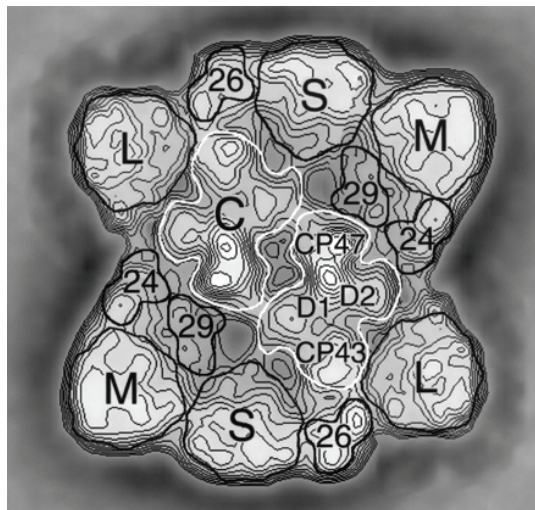
**Figure 3.** A general carotenoid excited state diagram; the solid arrow indicates the strongly allowed  $S_0 \rightarrow S_2$  transition while the dashed arrows indicate the forbidden transitions.

### Light-harvesting antennas

A number of chlorophylls and carotenoids are typically arranged in a three-dimensional structure to form a light-harvesting antenna. This system collects the light and transfers the excited-state energy to the reaction centre, a photochemical device where the electronic excited state is converted into a stable trans-membrane charge separation.

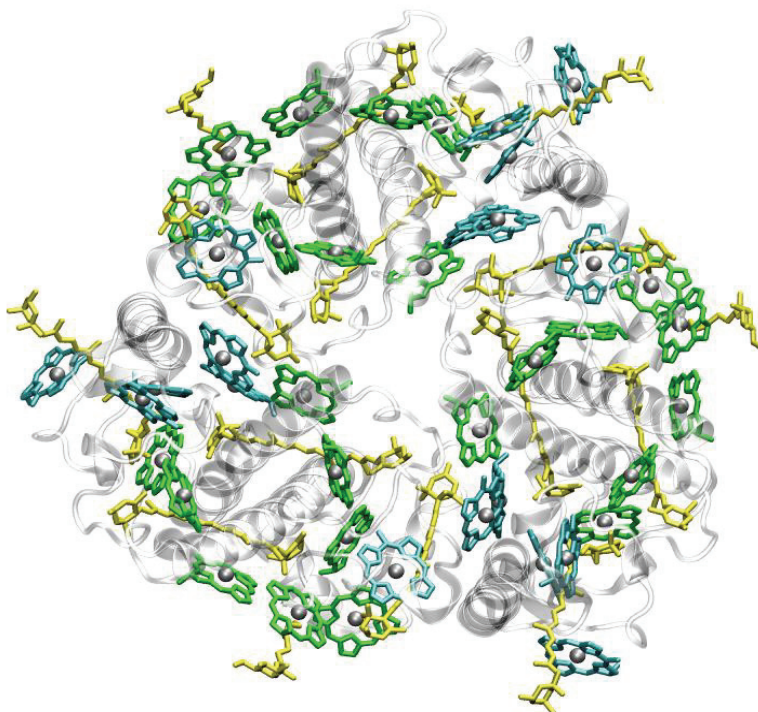
## The Light-Harvesting Complex II of Plants

The light-harvesting complex II (LHCII) is the most abundant membrane protein in the chloroplast and the major collector of solar photons in plants. Figure 4 shows the structural organization of LHCII in the photosystem II supercomplex as obtained by Boekema et al.<sup>14</sup>. LHCII binds with different strength to the PSII supercomplex as indicated by the labeling L (loosely bound), S (strongly bound) and M (moderately bound). As can be seen LHCII binds to the periphery of the PSII complex. Along with LHCII a number of minor antennas, CP49, CP24, CP26, and the core light-harvesting complexes CP47 and CP43 are also present in the complex. The light collected by LHCII is transferred either directly or via the minor antenna complexes to the PSII core (C) and finally to the PSII reaction centre, where the electron transfer reactions take place. LHCII also contributes to the partitioning of excitation energy delivered to PSI and PSII<sup>15</sup>. An extremely important and yet poorly understood role played by LHCII is in the photoprotection of the photosynthetic apparatus under conditions of excess light illumination. It was recently shown that LHCII has an inbuilt capacity to dissipate energy by switching from a light-harvesting, to an energy dissipating device<sup>16</sup>. In Chapter 5 a mechanistic model for energy dissipation is presented where under conditions of excess light LHCII undergoes a conformational switch by formation of aggregates, which opens up a channel for energy dissipation via a low-lying carotenoid excited state.



**Figure 4.** PSII supercomplex obtained by Boekema et al (1999). LHCII is bound with different strength to the periphery of the complex. The white line contour shows the PSII core. A number of minor light-harvesting antennas are also shown.

Figure 5 shows the crystal structure of LHCII in its trimeric form. In this structure each monomeric unit contains 8 Chl *a*, 6 Chl *b* and 4 carotenoids: 2 lutein, 1 violaxanthin and one neoxanthin molecule.

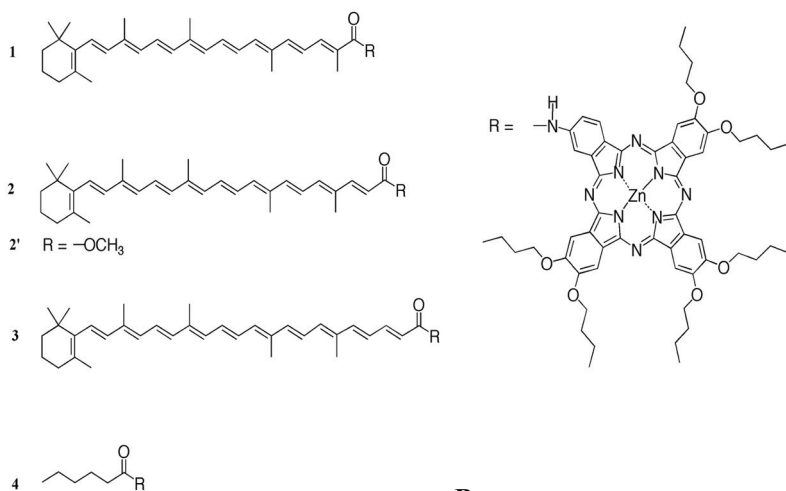


**Figure 5.** Crystal structure of the trimeric form of LHCII. In green are the tetrapyrroles of chlorophyll *a*, in cyan are the tetrapyrroles of chlorophyll *b*. The carotenoids are displayed in yellow.

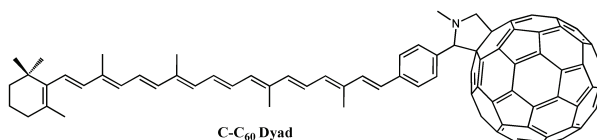
### Artificial light-harvesting antennas

In recent years the advances in chemical synthesis have made it possible to synthesize a number of biomimetic systems capable of reproducing a number of features of their natural counterparts<sup>17</sup>. The increasing interest in the area of artificial light-harvesting devices is manifold. Typically these systems are made up of a small number of chromophores arranged in a well defined three-dimensional structure. Understanding the photophysics and photochemistry of these systems and the underlying factors governing them can provide very valuable information to the understanding of the behaviour of the far more complicated natural systems.

A



# B



**Figure 6.** Two artificial light-harvesting devices studied in this thesis. (A) A zinc phthalocyanine molecule is covalently attached to a series of carotenoids of increasing conjugation length. (B) A fullerene C<sub>60</sub> molecule linked to a carotenoid which provides the system with an effective antenna molecule and electron transfer partner.



## Carotenoids in Photoprotection

A vital role played by carotenoids is in the photoprotection of the photosynthetic apparatus; when a chlorophyll molecule is excited to its singlet excited state there is a finite probability for the excited state to undergo intersystem crossing to populate the low-lying, long-living triplet state. This state is an excellent singlet oxygen sensitizer. Carotenoids are very efficient at quenching the chlorophyll triplet state by accepting energy from the latter:



The excited carotenoid can harmlessly dissipate its triplet excited state energy, being unable to sensitize singlet oxygen due to unfavourable energetics<sup>2,3</sup>. Carotenoids are also very efficient scavengers of singlet oxygen<sup>2,3</sup> thus minimizing damage to the photosynthetic organism:



Both properties rely on the low-lying carotenoid triplet state.

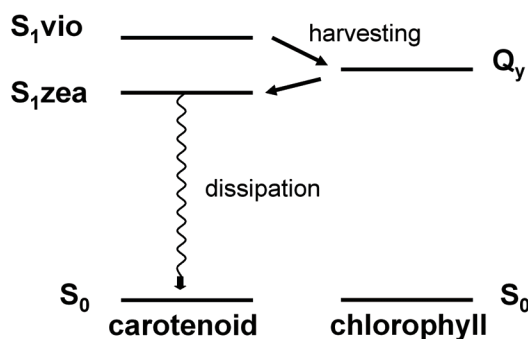
## Non-photochemical quenching

A very hot area in contemporary photosynthesis research aims at establishing the role of carotenoids in the quenching of chlorophyll excited singlet states under conditions of excess light illumination. When a plant is exposed to an amount of light that exceeds the amount required for maximum CO<sub>2</sub> fixation, a number of photoprotective mechanisms are activated to protect the plant from photooxydative damage that would derive from the accumulation of reactive oxidized and reduced species. The process is generally known as non-photochemical quenching (NPQ), since it leads to the quenching of chlorophyll fluorescence without formation of new chemical products<sup>18,19</sup>. Feedback deexcitation or energy dependent quenching (qE) is the major component of NPQ<sup>18,20</sup>. Its activation depends upon a number of factors: excess light illumination causes a drop in the thylakoid lumen pH which in turn triggers the xanthophyll cycle where violaxanthin, a 9 double-bond carotenoid, is converted into zeaxanthin, an 11 double-bond carotenoid<sup>21</sup>. As the intensity of light is decreased or under conditions of dim light the process is reversed. The drop in luminal pH also leads to the protonation of the PsbS protein<sup>22</sup>, a PSII subunit thought to have a central role in qE<sup>23</sup>.

## Proposed models for NPQ

### The molecular gear shift mechanism

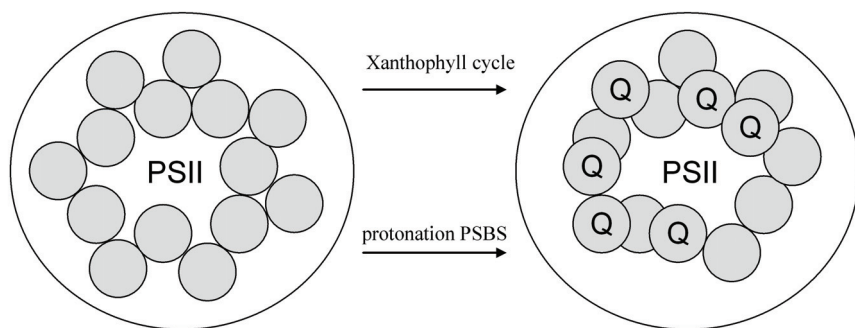
This model was proposed in 1994 by Frank and coworkers<sup>24</sup> and is based on the energetics of the xanthophyll cycle carotenoids: the increase in the conjugation length from 9 to 11 double bonds during the xanthophyll cycle, would turn the carotenoid low-lying singlet excited state from an energy donor into an energy acceptor as depicted in Figure 7. There has been a lot of speculation about this model and the literature is to date highly controversial. The main problem if one is to test the validity of the model is given by the fact that the states involved in energy transfer and fluorescence quenching are dark, i.e. invisible to conventional one-photon spectroscopy. Hence the notorious difficulties in establishing the exact positioning of the various energy levels with respect to the excited state of chlorophyll. In chapters 2 and 3 by making use of a series of simple artificial light-harvesting dyads we demonstrate for the first time the molecular gear shift mechanism in a model system by showing that the flow of energy from the carotenoid to the tetrapyrrole can be reversed upon addition of one double bond to the conjugated system of a carotenoid<sup>4,7</sup>. Alternatively, the increase in the conjugation length leads to a lowering of the oxidation potential of the carotenoid<sup>25</sup> and the quenching has been proposed to proceed via a charge separated state<sup>26</sup>. While the molecular gear shift model is very appealing for its simplicity and its elegance it is becoming increasingly clear that the simple substitution of violaxanthin with zeaxanthin cannot, by itself, account for NPQ<sup>27</sup>.



**Figure 7.** Schematic representation of the molecular gear shift mechanism: the increase in the conjugation length from violaxanthin to zeaxanthin turns the carotenoid  $S_1$  state from an energy donor into an energy acceptor.

## The aggregation model

First proposed in 1991 by Horton and coworkers<sup>28,29</sup>, in the aggregation model the xanthophyll cycle carotenoids are allosteric effectors that favour the transition of the main light-harvesting antenna (LHCII) into a quenched state. The essence of the model is depicted in Figure 8 while a more detailed description can be found in ref. 29. The xanthophyll cycle along with the protonation of the PsbS subunit promotes conformational changes of LHCII which, by their combined action, switches into a dissipative state. The transition from an unquenched state to a quenched state is an intrinsic property of each trimeric LHCII<sup>16</sup> which depending on the light conditions can work as a light-harvesting or as an energy dissipating device.



**Figure 8.** Simplified representation of the aggregation model: the xanthophyll cycle and the protonation of the PsbS protein promote conformational changes that turn LHCII into a quenched state (Q). The quenched LHCII acts as an energy sink.

## Energy transfer and excitonic interaction

Excitation energy transfer between pigments is the basic process within a light-harvesting antenna. How the energy is transferred among pigments depends on a variety of factors, among them the distance, the oscillator strength and spectral width of the transitions involved, their relative orientations, the overlap between wavefunctions, etc. Excitation energy transfer within an antenna system usually proceeds via Coulombic interaction between its pigments. In the case of two interacting pigments the Hamiltonian of the system can be written as:

$$H = H_D + H_A + V_{DA} \quad (4)$$

where  $H_D$  and  $H_A$  are the Hamiltonians of the isolated pigments and  $V_{DA}$  is the interaction term.

### Weak Coupling

If the donor and acceptor molecules are at a distance that is much larger than the size of the electron distribution within the individual chromophores, the interaction responsible for the excitation energy transfer between the two chromophores can be described by a long-range dipole-dipole coupling.

Thus, if the two molecules are uncharged, and their distance is large compared to the size of the molecules the interaction can be expressed by the dipole-dipole approximation, since dipole-dipole interaction is the dominant term in the multipole expansion of the interaction:

$$V_{DA} = \frac{\mu_D \cdot \mu_A - 3(\mu_D \cdot R_{DA})(\mu_A \cdot R_{DA})}{R_{DA}^3} \quad (5)$$

For weak interaction strengths the two molecules retain their spectroscopic identity and the energy transfer can be seen as a hopping process between the molecules as described by Förster<sup>30</sup>; the efficiency of energy transfer depends on the following three parameters:

- 1) the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor.
- 2) the distance between the pigments (the efficiency scales with the sixth power of the distance)
- 3) the relative orientation of the donor emission dipole and acceptor absorption dipole of the transitions involved in the energy transfer process.

The energy transfer rate is given by the following:

$$4\pi^2 \frac{V_{DA}^2}{\hbar^2} \cdot \frac{\int F_D(\nu) \epsilon_A(\nu) \nu^{-4} d\nu}{\int F_D(\nu) \nu^{-3} d\nu \int \epsilon_A(\nu) \nu^{-1} d\nu} \quad (6)$$

where  $F_D(\nu)$  is the emission spectrum of the donor D,  $\nu$  is the frequency,  $\epsilon_A(\nu)$  is the absorption spectrum of the acceptor A and  $V_{DA}$  is the interaction strength between the donor and the acceptor. The dipole-dipole approximation breaks down as the distance between pigments decreases and a certain amount of overlap between the electronic orbitals ensues. Under these conditions which typically apply when two chlorophyll molecules are at a distance of less than 1.5 nm the

molecules no longer see each others as oscillating dipoles and higher terms in the multipole expansion of the interaction have to be taken into account. Also, for larger values of the interaction energy the excitations must be viewed as truly delocalized over the interacting molecules and as a consequence the dynamics acquires a quantum mechanical character<sup>31</sup>.

### The exciton concept

The exciton concept, in combination with a model for relaxation, is commonly used to explain the spectroscopic properties and the energy transfer dynamics in molecular aggregates and biological structures<sup>32</sup>. The minimal system which can be used to illustrate the exciton concept is the so-called excitonically coupled dimer. The energy states of two non-interacting molecules are the solutions of the Hamiltonians:

$$H_1\psi_1^i = \varepsilon_1^i\psi_1^i \quad (7)$$

$$H_2\psi_2^i = \varepsilon_2^i\psi_2^i \quad (8)$$

As the two pigments are brought closer to each other the Coulombic interaction between them leads to a new term in the Hamiltonian.

If we take two identical molecules with fixed spatial orientation the solutions of the Hamiltonian:

$$(H_1 + H_2 + V)\psi^f = E^f\psi^f \quad (9)$$

are no longer the product of eigenstates of the Hamiltonian of the isolated molecules.

The solution of (9) is instead given by a linear combination of the product of the molecular eigenfunctions (within the Heitler-London approximation). After some manipulations (a detailed derivation can be found in ref. 32) one obtains that the solutions of the Hamiltonian have to satisfy the following:

$$\begin{vmatrix} \varepsilon_1^1 + V_{11} - E^f & V_{12} \\ V_{21} & \varepsilon_2^1 + V_{22} - E^f \end{vmatrix} = 0 \quad (10)$$

if the molecules are identical  $\varepsilon_1^1 = \varepsilon_2^1$ ,  $V_{12} = V_{21}$  and  $V_{11} = V_{22}$  which leads to:

$$(\varepsilon_1^1 + V_{11} - E^f)^2 = V_{12}^2 \quad (11)$$

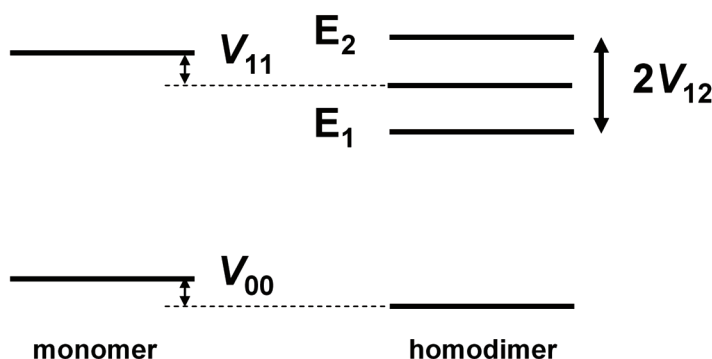
the solutions of (9) give the two eigenvalues:

$$E^1 = \varepsilon^1 + V_{11} + V_{12} \quad (12)$$

$$E^2 = \varepsilon^1 + V_{11} - V_{12} \quad (13)$$

thus the energy levels have been split by  $2V_{12}$  as schematically shown in Figure 9.

It can be shown that concomitantly with the appearance of new excited states, a shift in the ground state energy can also occur<sup>32</sup>. A schematic representation of the energy levels of an excitonically coupled dimer is shown in Figure 9.



**Figure 9.** Schematic representation of the energy levels of an excitonically coupled dimer.

## Experimental Techniques

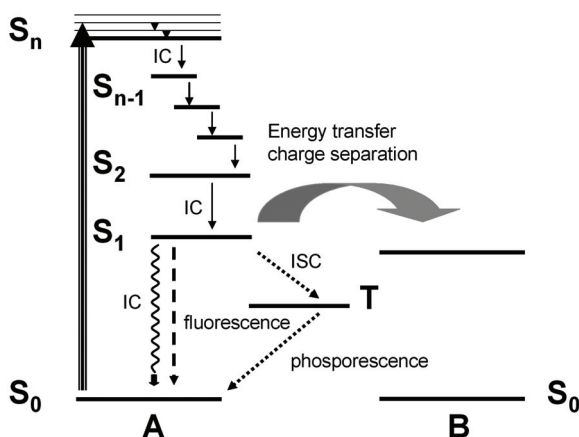
The very first reactions taking place after the absorption of a photon by a photosynthetic pigment are among the fastest known chemical reactions, taking place on a timescale ranging from tens of femtoseconds ( $1 \text{ fs} = 10^{-15} \text{ s}$ ) to a few nanoseconds ( $1 \text{ ns} = 10^{-9} \text{ s}$ ).

The advent of ultrafast titanium:sapphire oscillators capable of producing pulses of duration on the order of tens of femtoseconds in the early nineties opened up an entirely new and unexplored area of science by providing researchers with the tools necessary for the investigation of extremely fast photochemical, photophysical and photobiological reactions. The idea behind this kind of experiments is to populate the excited state of the system, (a pigment, a light-harvesting complex, etc) and probe the changes in light absorption or emission as a function of time. Most of the work presented in this thesis consists of time-resolved absorption spectroscopic

measurements. In the next sections a discussion of the technique employed for these experiments is presented along with the description of the experimental setup.

### Time-resolved spectroscopy

When a molecule is excited to one of its excited electronic states a number of processes can take place as shown in Figure 10. From an higher excited state level ( $S_n$ ) the excitation goes down the vibrational and electronic excited state ladder to populate the lowest excited state of the same multiplicity. From that state, depicted as  $S_1$  in the figure, a number of competing processes can take place. The molecule (A) can transfer energy to a nearby molecule (B) or a charge separated state can be formed (in some cases, for instance for a number of carotenoids in light-harvesting systems, energy transfer and possibly charge separation can take place from the second singlet excited state as well). Alternatively, the excited  $S_1$  state can undergo intersystem crossing to populate a triplet state or can lose its energy by decaying directly to the ground state via internal conversion, i.e. the excited state energy is dissipated as heat, or radiatively, i.e. giving rise to fluorescence. The triplet state can slowly decay to the ground state by emitting a photon (phosphorescence) or its energy can be dissipated as heat.



**Figure 10.** The fate of an electronically excited state.

We have seen in Figures 1 and 2 that chlorophylls and carotenoids have a distinct and unique absorption spectrum. If one could determine how the absorption properties of the various pigments change as a function of time, for instance when

they are embedded in a light-harvesting device, one could establish whether energy transfer or charge separation has taken place within the system, how much energy was lost and on which timescale the various processes occurred. This is accomplished by making use of ultrafast spectroscopy.

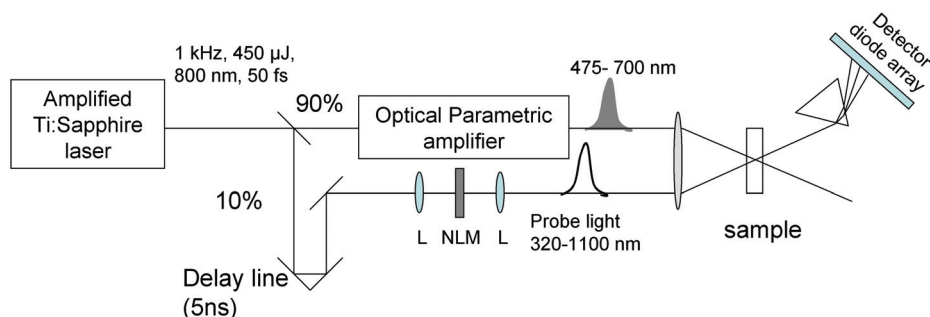
The absorbance, or optical density (*OD*) of a sample is defined as:

$$OD(\lambda) = -\log \frac{I(\lambda)}{I_0(\lambda)} \quad (14)$$

If some of the molecules within the sample are promoted to an excited state by pumping the sample with an excitation beam, one can look at the difference in the absorbance between the pumped (pump on) and unpumped (pump off) system to determine the following quantity:

$$\Delta OD(\lambda, t) = OD(\lambda, t)_{on} - OD(\lambda)_{off} = \log \frac{I(\lambda)_{off}}{I(\lambda, t)_{on}} \quad (15)$$

Figure 11 shows a schematic representation of the experimental setup. The output of a titanium:sapphire laser system is split into two beams. About 90 % of the energy is used to drive an optical parametric amplifier where the pump beam is generated. The remaining 10 % of the energy is sent through a delay line and focused on a non-linear medium (BBO) to generate a white light continuum. The pump and probe beams are then focused on the sample, normally contained in a 1 mm cuvette, after which the probe light is dispersed and collected by a diode array while the pump beam is blocked. The sample is normally mounted on a shaker or on a flow cell to make sure that it is refreshed after each laser shot during the experiment.



**Figure 11.** Schematic representation of the experimental setup.



## Data Analysis

A typical time-resolved experiment consists of a collection of thousands of datapoints, i.e tens to hundreds wavelengths times one to two hundred timepoints. In order to be able to extract valuable information one could simply take slices of the data; for instance one could take one wavelength and look at the evolution in time, that is defined as a kinetic trace; or one could select one timepoint and select a spectrum, i.e the signal at all wavelengths at a fixed time delay. This is normally the first step in the data analysis in an attempt to get a glimpse of an expected (or unexpected) evolution. However, trying to extract as much information as possible in this way would prove a Sisyphean task. The experimenter would like to be able to distill the overwhelming amount of data into a relatively small number of spectra and parameters in order to make a first attempt at interpreting the dynamics and kinetics of the experiment. This is done by making use of global analysis techniques for data analysis<sup>33</sup>. In global analysis the data are described by a number of compartments with specific spectral properties connected by linear rate kinetics. At time zero the excitation sits on compartment 1 which decays to compartment 2 with a rate constant  $k_1$ . The second compartment is thus populated with a rate constant  $k_1$  and at the same time is depopulated with a rate constant  $k_2$ . Thus the evolution of the population of the various compartments is described by the following differential equations:

$$dC_1(t)/dt = I(t) - C_1(t)k_1 \quad (16)$$

$$dC_i(t)/dt = k_{i-1}C_{i-1}(t) - k_iC_i(t) \quad i \neq 1 \quad (17)$$

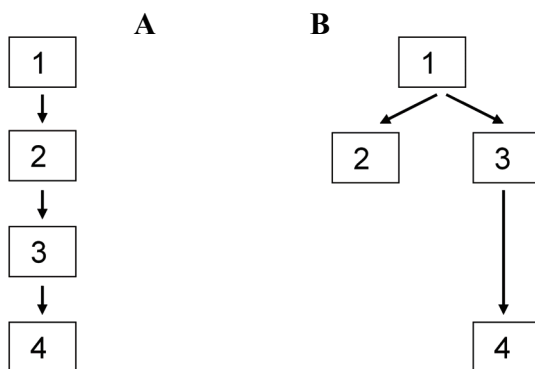
thus the excitation pulse  $I(t)$  populates the first compartment, which in turn decays to the second compartment with a rate constant  $k_1$ .

In this kind of analysis the number of compartments is increased until the addition of an extra compartment does not lead to an improvement of the quality of the fit, which can be assessed by the shape of the residuals matrix associated to the fit. Thus for each compartment one obtains a rate constant and a spectrum. The evolution of the data can thus be described by the following sum:

$$F(t, \lambda) = \sum_i C_i(t) \sigma_i(\lambda) \quad (18)$$

The global analysis with the sequential model just described can be very insightful. The obtained spectra, evolution associated different spectra (EADS) can be regarded as an average state of the system that evolves in time and in general represent a mixture of "pure" molecular states. From the same analysis a number of time constants are obtained which also correspond in the most general case, to a

weighted average of the time constant associated to a number of processes. After a detailed analysis of the raw data with the help of a global analysis, and if the amount of information contained in the latter suffices, one typically takes the analysis one step further as an attempt to extract the species associated difference spectra (SADS), *i.e* the pure molecular spectra involved in the various processes. At this stage the sequential model is branched in such a way to reflect the true underlying photophysics or photochemistry of the system. An illustration of the global and target analysis for a 4-compartment system is shown in Figure 12.



**Figure 12.** Schematic representation of global (A) and target (B) analysis model for a 4-compartment system.

## This Thesis

The topics covered by this thesis range from artificial to natural light-harvesting systems. In chapters 2 and 3 a thorough investigation of the photophysics of a series of artificial caroteno-phthalocyanine (Car-Pc) light-harvesting antennas is presented. When the Pc moiety is selectively excited the attached carotenoid is capable of strongly quenching the Pc excited state; remarkably the process is very sensitive to the conjugation length of the carotenoid, the addition of one double bond to the conjugated chain being able to turn the carotenoid from a non-quencher into a very strong quencher. We show that the underlying quenching mechanism is energy transfer from the excited phthalocyanine to a low-lying carotenoid excited state. In chapter 3 it is shown that the system is able to reproduce several light-harvesting properties of carotenoids observed in various natural light-harvesting antennas. The carotenoid to Pc energy transfer efficiency is strongly dependent on the conjugation length of the carotenoid reaching an efficiency near unity for a nine-double bond carotenoid. In the same chapter we also show a new phenomenon

involving the somewhat mysterious S\* state. Chapter 4 presents an extensive investigation of the photophysics and photochemistry of a caroteno-C<sub>60</sub> dyad. When the system is dissolved in the polar solvent toluene we detected a multiphasic charge separation upon excitation of the carotenoid molecule. The charge separation channels are precluded when the system is dissolved in hexane, a non-polar solvent.

In chapter 5 a mechanistic model for non-photochemical quenching in plants is proposed. For the first time the molecular mechanism underlying qE has been detected: energy transfer to a low-lying carotenoid excited state. In chapter 6 an extensive investigation of the role of carotenoids in light-harvesting and chlorophyll fluorescence quenching in aggregates of the cyanobacteria light-harvesting system, IsiA is presented. In the light of the results presented in chapters 2, 5 and 6 we propose a general mechanisms for the quenching of chlorophyll *a* fluorescence by carotenoids, which may have been adopted by different photosynthetic organisms to cope with the deleterious effects of excess light.

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